

# THE UNIVERSITY OF SHEFFIELD

## REPORT OF EXAMINERS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

### FACULTY OF SCIENCE

This Report should be completed by the Examiners and returned, together with the Examiners' Preliminary Reports, to the Faculty Administrator at Research & Innovation Services (231 Glossop Road, Sheffield, S10 2GW). Before completing the Report form, please read the attached *Guidance Notes for Examiners*. Please note that the examiners are responsible for providing the candidate with details of any required amendments as soon as possible following the viva examination.

The candidate (and supervisor on request) will receive a copy of the final Report (comprising the preliminary and joint Reports), which may be released by Research & Innovation Services only, following formal approval by the Vice-Chancellor acting on behalf of the Senate.

Name of candidate: Qiang Wan Registration Number: 090240388

Department: Department of Molecular Biology and Biotechnology

Name of Supervisor: Professor P A Bullough

Title of Thesis: Structure and assembly of bacillus spore proteins

Names of Examiners: External Dr E Morris  
Internal Dr S Mesnage

Name of coordinator:

### SECTION A: Specific Recommendation

We, the undersigned, make the following recommendation (please tick **one** box only):

1. ☐ that the degree of PhD be awarded without the need for any corrections to the thesis.
2. ☒ that the degree of PhD be awarded once specified minor corrections have been completed to the satisfaction of the Internal/External Examiner (please return this form as soon as possible after the examination and sign and return the enclosed minor corrections form ONLY when the specified examiner is satisfied that the amendments are complete. Please ensure that the candidate receives details of the required amendments as soon as possible.
3. ☐ that the degree of PhD be not now awarded, but that the candidate be allowed to undergo a further oral examination without modification of form or content of the thesis.
4. ☐ that the degree of PhD be not now awarded, but that the candidate be allowed to submit a revised thesis after such modification of form or content as the Examiners may prescribe, WITH / WITHOUT\* oral re-examination. (\*Please delete as appropriate).
5. ☐ that the degree of PhD be not now awarded, but that the degree of Master of Philosophy be awarded (subject only to the necessary changes to the cover and title page of the thesis).
6. ☐ that the degree of PhD be not now awarded, but that the candidate be allowed to submit a revised thesis for the degree of Master of Philosophy after such modification of form or content as the Examiners may prescribe, WITH / WITHOUT ORAL\* re-examination. (\*Please delete as appropriate)
7. ☐ that the degree of PhD be not awarded.

Name of Examiner: Edward MORRIS

Signed:



Date: 22-04-2013

Name of Examiner: Stéphane MESNAGE

Signed:



Date: 22-04-2013

### For office use only

DDP/RTP completed:  
(where applicable):

☐

Viva Date:

☐

Ethics Approval:

☐

eThesis uploaded: (08 registration  
numbers onwards)

☐

CAS student:

☐

Time Limit:

Approved by Faculty

Date:

Signed:

## SECTION B: Report on the Thesis

1. Give below a clear indication of the subject matter of the thesis and your assessment of it. This should be a sufficiently detailed statement to justify the specific recommendation made in Section A.

This thesis deals with the structural characterisation of coat and exosporium proteins from spores originating from different *Bacillus* species. Using negative stain or cryo-EM methodologies, the candidate analysed several samples corresponding to (i) two-dimensional crystalline arrays or (ii) single particles made of recombinant proteins produced in *Escherichia coli* and (iii) purified exosporium fragments purified from intact spores.

The results described in the manuscript represent a significant contribution in the field and will certainly form the basis of one or more good publication in peer-reviewed journal(s). The quality of the work is of a high standard and has been carried out rigorously.

The manuscript is in general adequately organized and presented. However, the standard of English and grammar is rather poor and there are several mistakes of this nature that require correction. In numerous sections, the candidate could have given a more thorough explanation of the theoretical background of electron microscopy and image analysis.

The modifications to carry out have been notified to the candidate.

2. Are you satisfied that the thesis is the candidate's own work or, if it was done in collaboration, that his or her share in the research is adequate?

YES

3. Is the mode of presentation of the thesis satisfactory?

YES

4. Is the thesis an addition to knowledge and worthy of publication either in full or in abridged form (such as a paper or papers in an appropriate journal)?

YES

5. Can the thesis be viewed as a coherent piece of work as defined in the *Guidance Notes for Examiners*?

YES

6. Please use the space below if you wish to make any comments on the appropriateness of the supervision/support that the student has received whilst undertaking the research and preparing the thesis. (Optional).

No particular comment to make.

## SECTION C: Report on the Oral Examination

Date of oral examination: 22<sup>nd</sup> April 2013

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1. Do you consider the candidate to be adequately acquainted with the field of knowledge within which the subject matter of the thesis falls?

YES.

2. General comments on the oral examination, e.g. the candidate's overall performance and ability to defend the thesis

Wan performed well in the viva and was able to answer questions on a wide range of topics both directly and indirectly linked to his thesis work. He generally demonstrated a good understanding of the background biology and techniques used in his work, and was able to explain concepts well with minimal prompting. The examiners both agreed that the recommendation was to award a PhD with minor corrections and these were discussed with the student.

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## SECTION D: Additional general remarks or recommendations (if any)

No additional remarks or recommendations.

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## PRELIMINARY REPORT OF EXAMINERS

### FACULTY OF SCIENCE

Each Examiner is required to complete and bring to the oral examination an independent preliminary report on the thesis. The Preliminary Report will be appended to the Examiners' Joint Report. It would be helpful if the Report could be typewritten. Before completing the Report form, please read the attached *Guidance Notes for Examiners*

The candidate (and supervisor on request) will receive a copy of the final Report (comprising the preliminary and joint Reports), which may be released by Research & Innovation Services only, following formal approval by the Vice-Chancellor acting on behalf of the Senate.

Name of candidate: Qiang Wan  
Registration Number: 090240388  
Department: Department of Molecular Biology and Biotechnology  
Name of Supervisor: Professor P A Bullough  
Title of thesis: Structure and assembly of bacillus spore proteins  
Names of Examiners: Internal Dr S Mesnage  
External Dr E Morris

Name of Coordinator  
(where applicable):

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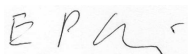
Please give below a report on the thesis, using additional sheets as necessary. No specific recommendation concerning the award of the degree should be given at this stage.

This thesis describes the structural characterisation of the coats and coat proteins from a number of different species of bacterial spores. Proteins were expressed in *E coli* and purified and the coat assemblies were prepared from a number of different types of spore. Structural characterisation was made by electron microscopy and image processing using both negative stain and cryo-EM methodologies. Most of the image analysis involved the two-dimensional crystal approach, while some analysis was carried out by the single particle technique. The thesis contains a number of interesting and novel observations which would form the basis of one or more good publications. The work is of a high standard and is generally well documented. However, the standard of English and grammar is rather poor and there are numerous mistakes of this nature that require correction. There are a number of areas where the candidate could have given a more thorough explanation of the theoretical background of electron microscopy and image analysis. Assuming the candidate performs well in the exam and carries out the appropriate corrections to the thesis he should comfortably meet the standard for a PhD.

Name of Examiner: Dr Ed Morris

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Signed:



Date:

22 April 2013

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## PRELIMINARY REPORT OF EXAMINERS

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External Dr E Morris

Name of Coordinator

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Please give below a report on the thesis, using additional sheets as necessary. No specific recommendation concerning the award of the degree should be given at this stage.

The thesis describes work dealing with the structural analysis of proteins found in the spore coat and exosporium of several *Bacillus* species.

The introduction is a review of the field and gives the necessary background information. The materials and methods cover all the techniques used and are sufficient to allow the repetition of the work. There are four results chapters. The first chapter describes the purification of several recombinant *B. subtilis* spore coat proteins (CotE, CotH, CotV, CotW, CotY, CotZ, SpoIVA, SpoVID, SafA,) and a *Bacillus cereus* exosporium protein (ExsY), and their preliminary analysis by electron microscopy. The following chapters of the results deal with the electron crystallography and single particle analysis of crystals formed by some of the proteins previously studied: CotY, SafA and CotV-W complexes (second chapter), and ExsY (third) chapter. The fourth and last chapter of the result section describes the structural analysis of exosporia from several *Bacillus*. Two samples from *Bacillus circulans* 42G1 and *Bacillus fusidiformis* ATCC7055 were chosen for a detailed structural analysis by electron crystallography and cryo-electron microscopy.

The final discussion chapter provides a critical analysis of the results in the light of the data available in the field.

Although the manuscript would require significant editing to correct typographical mistakes and minor problems with the content of some sections, the thesis is in general adequately organized and presented. It contains a significant amount of experimental work. The results presented in the manuscript will undoubtedly lead to a publication in a very good peer-reviewed journal in the short term, and contribute to other publications in a longer term.

The knowledge and understanding of the candidate will be tested during the oral examination.

Name of Examiner: Stéphane MESNAGE

Signed:



Date: 22/04/2013

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